Composition and Method

Background of the Invention

Maintaining the well being of the GI tract of a mammal is a very desirable goal. Particularly annoying are inflammatory conditions of the GI tract. Some of the signs of inflammation of the GI tract include acute or chronic diarrhea, soft stools, blood in stool, vomiting, poor nutrient digestion and absorption, weight loss and poor appetite. Diseases such as gastritis, enteritis, colitis, inflammatory bowel disease, ulcers, certain types of cancer and other conditions are known to have GI inflammation as a main component.

We have found that a mixture of certain materials can bring about the amelioration of the principle signs of GI inflammation such as diarrhea. The frequency of eliminations as well as the quality of the elimination can be substantially improved when GI tract inflammation is improved, particularly in a companion pet such as a cat, when appropriate levels of glutamine, fermentable fiber(s), antioxidant(s) and omega (n)-3 fatty acids are orally administered to the mammal.

Summary of the Invention

In accordance with the invention, there is a composition suitable for mammalian oral ingestion in a mammal having GI tract inflammation comprising an anti-diarrhea effective amount of a combination of glutamine, fermentable fiber(s), antioxidant(s) and omega-3 fatty acid(s).

A further aspect of the invention is a method for managing diarrhea in a mammal having GI tract inflammation comprising orally administering to the mammal a composition described above.

Detailed Description of the Invention

Glutamine is a well known as a material which is important for lymphocytes to proliferate and important as a nutrient for intestinal cells. Glutamine is also a precursor for glutathione, a

natural antioxidant in the body. All wt % disclosed here for any constituent are on the basis of a daily diet for the mammal. All numbers are calculated on a dry matter basis.

The quantity of glutamine is a minimum of about 0.1, 0.15 or 0.2 wt %. The maximum generally does not exceed about 5, 4 or 3 wt %.

Fibers which can be employed are those which are moderately fermentable, highly fermentable or blends of the two. Low or non-fermentable fibers can also be added at low levels without impacting the formulation.

We have shown that certain prebiotic fiber ingredients when fermented by existing bacteria from the GI tract of dogs and cats produce high levels of butyrate and other short chain fatty acids which would acidify the GI tract and reduce the growth of pathogens. Prebiotic fibers that produce high levels of butyrate include but are not limited to mannan-oligosaccharide, pectin, xylooligosaccharide, burdock, beet pulp, inulin, galactose, other xylans, fructans, dextrans, beta glucan, resistant starches, polysaccharide from gums, etc., should be present at levels between about 0.5 – 20 wt % of diet with the preferred levels between about 1 – 5 wt %. Gums may include gums produced by microorganisms such as gellan gum, xanthan or gums produced by plants such as acacia. The blend is preferably formulated based on high butyrate production and moderate fermentability based on volatile fatty acids (VFA) production and organic matter disappearance to help maintain optimal GI health. The composition can include at least about 10 – 60% of a moderately fermentable fiber and about 20 – 40% of a highly fermentable fiber. These fibers should be chosen such that the butyrate production of these fibers is high, between about 5 – 40% of total VFA. Moderately fermentable fibers are defined as having an organic matter disappearance of from about 15 to 60 percent when fermented by fecal bacteria in vitro for a 24 hour period. That is, from about 15 to 60 percent of the total organic matter originally present is fermented and converted by the fecal bacteria. Highly fermentable fibers have greater than a 60 % disappearance rate.

Antioxidants can also be employed in the compositions and methods. Vitamin E, C and blends thereof can be employed. Any precursors of these vitamins can be employed, such as tocopheryl acetate and sodium ascorbate. Vitamin E is a minimum of about 0.1, 0.2 or 0.4 wt % and generally does not exceed a maximum of about 3, 2 or 1 wt% of the diet. Vitamin C is a minimum of about 0.1, 0.2 or 0.4 wt% and generally does not exceed a maximum of about 3, 2, or 1 % of the diet.

Omega-3 fatty acids are well known dietary constituents and are primarily found in fats and oils, particularly fish oils such as menhaden, salmon and the like. Principle constituents of the omega-3 fatty acid are ecosapentaenoic acid (EPA), docosahexanoic acid (DHA) and alpha-linolenic acid (ALA). The quantities of omega-3 fatty acids are generally a minimum of about 0.1, 0.2 or 0.5 wt % and generally do not exceed a maximum of about 3, 2 or 1 wt %. Also generally present in the fats and oils are omega-6 fatty acids. The proportion of omega-6 fatty acid when present to omega-3 fatty acid on a weight basis is from about 0.5:1 to 6:1, preferably about 2:1 to 4:1.

The following examples illustrate the benefits to be achieved using the composition of the invention in managing diarrhea in a mammal. The mammal has or can have GI tract inflammation, preferably inflammatory bowel disease.

Example 1

In the following study, 12 cats with inflammatory bowel disease (IBD) were fed 2 varieties of food for a period of 2 weeks each. Six cats were fed Food A and 6 cats fed Food B for 2 weeks, followed by a crossover. Stool quality was monitored daily and the score based on a 1-5 scale, with 1 being runny and watery and 5 being hard and formed, see scores below. Stools from cats with IBD typically are 1 or 2.

Stool Monitoring Scoring

1: watery

2: soft, unformed

3: soft, formed, moist

4: hard, formed, dry

5: hard, dry pellets

Table 1 shows the effect of diets on the stool quality of cats with chronic diarrhea. The table show the percent of stools with scores of 1-5. The first canned Food A contained 3% of a

fiber with low fermentability, less than 15%, and the canned Food B contained 1.5% of a fiber with high fermentability, above about 60% fermentability. The nutrient content of the foods are listed below.

	Food A	**Food B**
	Low fermentable fiber food	**High fermentable fiber food**
Moisture	**72.69**	**72.58**
Protein-Kjeldahl	**8.24**	**7.94**
Fiber, Crude	**0.3**	**0.2**
Crude fat by acid hydrolysis	**9.58**	**9.85**

Results

The results show that feeding Food B containing a highly fermentable fiber source improved the stool quality of the cats from having 42% stools scoring 1's and 2's to only 15% scoring 1's and 2's.

Table 1

	% of Stools	
	Food A	**Food B ***
Stool quality Score	**Low fermentable fiber food**	**High fermentable fiber food**
1	11	2
2	31	13
3	41	45
4	10	22
5	7	14

* *4% of stools were not available for grading*

Example 2

Table 2 shows the data from a study where the same cats as in example 1 were fed 2 different foods. Both foods contained similar levels of prebiotic fibers and Omega-3 fatty acids. Food C contained added glutamine and antioxidants whereas Food D did not contain added glutamine or antioxidants. Half the cats were fed Food C for 2 weeks and the other half were fed Food D. This was followed by a washout of one week for all the cats. They were then crossed over to the other

food for an additional 2 weeks. The results in Table 2 show that when the cats were fed Food C that included glutamine and high antioxidants, the stool quality was significantly improved (0% stool score of 1 and 2) compared to the stool quality when the cats were fed Food D, the diet without added glutamine and antioxidants (7% stool with score of 1 and 2). Food C has significantly better results in stool quality, 0% stool scores of 1 and 2, compared to Food A having 42% of its stool score 1 and 2. Food C is also significantly better than Food B having 15% of stool scores of 1 and 2. Food C is also significantly better than Food D which has 7% of stool scores 1 and 2. Food C has all the significant components of this invention: glutamine, antioxidant, fermentable fiber and n-3 fatty acids. Foods A, B and D were all missing at least one of these components.

The nutrient content of the food is listed below.

Formula	**All options (Food C)**	**All options except glutamine and antioxidant (Food D)**
Moisture %	75-76	75-76
Protein-Kjeldahl %	10	10.1
Crude Fiber %	0.2	0.4
Ash %	1.49	1.69
Crude Fat %	4-6	4-6
Insoluble fiber %	1-1.5	1-1.5
Soluble fiber %	0.1-0.3	0.1-0.3
Omega 3 (calc)	0.13	0.06
Omega 6 (calc)	1.51	0.46
ascorbic acid µg/g	30-50	4-10
total tocopherols µg/ml	300-400	30-50

Table 2 .

Percentage of Stools

Stool quality score	Food C [all options]	Food D [all options except glutamine & antioxidants]
1	0	0
2	0	7
3	29	67
4	58	27
5	13	1

The data shows that the diet with added glutamine and antioxidants continues to sustain the improvement in stool quality in these cats.

Example 3

The next experiments show that the glutamine source that was used in the previous example is bioavailable and is able to stimulate the immune function. Glutamine is an important nutrient to the intestinal tract as it is the major fuel source for enterocytes and lymphocytes. A majority of the glutamine in the diet is absorbed by cells of the intestine as well as immune cells in the intestine .

In one experiment, a source of glutamine was tested to see if it was bioavailable and able to deliver adequate glutamine to the intestinal cells. The source of glutamine was a wheat hydrolysate with an enrichment of 30% glutamine. A dose response study was carried out in 6 dogs to see if increasing levels of the glutamine source (0, 0.5, 1.0, 2% glutamine content) was detected in the plasma after feeding the diet.

Table 3

Change in postprandial plasma glutamine in animals fed foods supplemented with different levels of glutamine.

% supplemented glutamine	%Change in plasma glutamine from control
0.5%	3
1.0%	10
2.0%	15

The data shows that there was an increasing response to the increased levels of glutamine in the diet, particularly 30 min after the meal. This shows that the glutamine is available to the blood stream after extraction by the intestinal cells.

In a further experiment, the efficacy of glutamine as a immune-modulator was examined. 20 Beagle dogs were randomly allocated into 4 groups receiving either basic diet or basic diet supplemented with 1%, 2%, or 4% glutamine. Blood samples were drawn in heparinized tubes from animals 2 hrs after their last feeding on day 1 and 16. Samples were prepared for immune measurement. (T cell proliferation assay).

T-cell proliferation assay. Peripheral blood leukocytes (PBL) in each blood sample were counted using Nova Celltrak II (Beckman Coulter Corp., FL). Blood was diluted (1:20) with supplemented media. Diluted blood was plated, in triplicate, in 96 well cell culture plates with the following mitogens diluted in supplemented media: Concanavalin A (0.5µg/ml, 2.5µg/ml, and 10 µg/ml), PWM (1.5µg/ml, 2.5 µg/ml), and PHA (0.5 µg/ml, 2.5 µg/ml. Plates were incubated in a humidified incubator containing 7% CO_2 at 37°C for 72 hrs. Cellular DNA was Ci/ well [□pulse-labeled 18 hrs before harvesting with 1 ^{3}H] thymidine. Cellular DNA was harvested on glass fiber paper using a cell harvester (Skatron Instruments Inc., VA) and suspended with 1.5 ml scintillation cocktail. [^{3}H]thymidine uptake was quantified as counts per minutes (CPM) using TriCarb 2100TR Liquid Scintillation Analyzer (Packard

BioScience Company, IL). Counts were normalized to CPM/10,000 cells to account for variation in PBL concentrations.

Effect of glutamine on lymphocyte proliferation

Concanvalin A (Con A) is a polyclonal T-cell mitogen. In the presence of Con A mitogen, overall analysis showed a significant effect of diet, but no effect of Con A dose, or dietary treatment by Con A dose interaction. Thus, the data were collapsed across the different doses of Con A to show the proliferative response of lymphocytes dependent on percentage of glutamine supplemented in the diet.

Table 4: Proliferation of T cell lymphocyte in response to ConA mitogen

Food	**T cell proliferation ($\log_{10}$ cpm)**
No supplemented glutamine	4.7
1% supplemental glutamine	5
2% supplemental glutamine	4.8
4% supplemental glutamine	4.5

There was a significant main effect of dietary treatment ($P < 0.01$). Dietary supplementation of 1% glutamine showed maximum lymphocyte proliferation which was significantly different from the control group ($P < 0.05$). Dogs supplied with 1% and 2% glutamine showed similar increases in lymphocyte proliferation. There was a significant difference between these groups and the proliferative response of lymphocytes from animals supplemented with 4% glutamine in their diet ($P < 0.01$). This indicates that supplementation with 1-2% glutamine enhances overall T-lymphocytes proliferation. However, 4% glutamine is not additionally beneficial in this respect.